Amendments to the Claims

This listing of claims will replace all prior versions of claims in the application.

Listing of Claims

What is claimed is:

1. (Currently Amended) A method for generating a secreted ~~soluble~~ disulfide bond-linked trimeric fusion protein, comprising:
(a) creating a DNA construct comprising a transcriptional promoter linked to a template encoding a fused protein subunit comprising a signal peptide sequence followed by in-frame fusion to a non-collagenous polypeptide comprising a ligand binding domain ~~to be trimerized~~, which in turn is joined by in-frame fusion to a mammalian polypeptide ~~capable of self-trimerization~~ which is heterologous from the non-collagenous polypeptide ~~to be trimerized~~ and which is capable of self-trimerizing said fused protein subunit to form said disulfide bond-linked trimeric fusion protein containing three ligand binding domains, wherein said trimeric fusion protein has an increased binding affinity to a ligand than a monomeric ligand binding domain (b) introducing said DNA construct into a eukaryotic cell; (c) growing said host cell in an appropriate growth medium under physiological conditions to allow said fused protein subunits to trimerize into the disulfide bond-linked trimeric fusion protein and to further allow the secretion of ~~a~~ the trimeric fusion-protein ~~encoded by said DNA sequence~~; and (d) isolating said secreted trimeric fusion protein from the culture medium of said host cell.

2. (Currently Amended) The method of claim 1 wherein the disulfide bond-linked trimeric fusion protein is a homotrimer.

3. (Currently Amended) The method of claim 1 wherein the mammalian polypeptide ~~capable of self-trimerization~~ comprises the C terminal portion of collagen capable of self-assembly into a trimer. ~~selected from the group consisting of pro.alpha.1(I), pro.alpha 2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI).~~

4. (Canceled)

5. (Canceled)

6. (Currently Amended) The method of any one of claims 1-3, wherein the signal peptide sequence and the non-collagenous polypeptide ~~to be trimerized~~ are both from the same native secreted protein.

7. (Currently Amended) The method of any one of claims 1-3, wherein the signal peptide sequence and the non-collagenous polypeptide ~~to be trimerized~~ are selected from two different secreted proteins.

8. (Previously Presented) The method of claim 1, wherein the host eukaryotic cell is a fungal or insect cell.

9. (Previously Presented) The method of claim 1, wherein the host eukaryotic cell is a cultured mammalian cell line.

10. (Previously Presented) The method of claim 3, wherein the C-terminal portion of collagen includes a "glycine-repeat" triple helical region of collagen linked to a C-propeptide.

11. (Currently Amended) The method of claim 3, wherein the C-terminal portion of collagen is encoded ~~identified~~ by ~~SEQ ID NOS:1-2~~ SEQ ID NO:1.

12. (Currently Amended) The method of claim 3, wherein the ~~trimerizing~~ C-terminal portion of collagen comprises only a C-propeptide without any glycine-repeat triple helical region of collagen.

13. (Currently Amended) The method of any one of claims 10-12, wherein the ~~trimerizing~~ C-terminal portion of collagen comprises a mutated or deleted BMP-1 protease recognition sequence, thereby conferring the trimeric fusion proteins resistance to BMP-1 protease degradation.

14. (Currently Amended) The method of claim 12 or 13, wherein the ~~trimerizing~~ C-terminal portion of collagen is encoded ~~identified~~ by ~~SEQ ID NOS:3-4~~ SEQ ID NO:3.

15-19. (Canceled)

20. (New) The method of claim 3 wherein the C terminal portion of collagen is selected from the group consisting of pro.alpha.1(I), pro.alpha 2(I), pro.alpha.1(II), pro.alpha.1(III), pro.alpha.1(V), pro.alpha.2(V), pro.alpha.1(XI), pro.alpha.2(XI) and pro.alpha.3(XI).

21. (New) The method of claim 20, wherein the C-terminal portion of collagen has the amino acid sequence shown as SEQ ID NO:2.

22. (New) The method of claim 12 or 13, wherein the C-terminal portion of collagen has the amino acid sequence shown as SEQ ID NO:4.

23. (New) The method of claim 1, wherein the non-collagenous polypeptide is soluble TNF alpha receptor or functional portion thereof.

24. (New) The method of claim 23 wherein the soluble TNF alpha receptor is soluble human TNF alpha receptor or functional portion thereof.

25. (New) The method of claim 23, wherein the soluble TNF alpha receptor is selected from the group consisting of soluble p55 TNF alpha receptor and soluble p75 TNF alpha receptor.

26. (New) The method of claim 1, wherein the ligand is TNF.

27. (New) The method of claim 1, wherein the non-collagenous polypeptide is soluble CD4 receptor or functional portion thereof.